

Replace the paragraph beginning at page 5, line 4, with the following rewritten paragraph:

C3
--The invention provides an isolated nucleic acid that encodes three start codons; each start codon is located within one of the three reading frames. The start codons can be ATG codons and can be found within a span of 50 nucleotides. In one embodiment, the nucleic acid encoding the three start codons has the sequence 5' ATGGCATGGCATG 3' (SEQ ID NO:18). The isolated nucleic acid that encodes the three start codons also can have a ribosome-binding site positioned 5' of the start codons.--

Replace the paragraph beginning at page 5, line 10, with the following rewritten paragraph:

C3
--In another embodiment, the invention provides for a vector that has a portion that encodes three start codons, one in each reading frame. The start codons can be ATG codons that occur within a span of 13 nucleotides, and more specifically, the 13 nucleotides can have the sequence 5' ATGGCATGGCATG 3' (SEQ ID NO:18). Furthermore, the vector that has a portion encoding three start codons also can have a portion that encodes histidine tags in three reading frames. In addition, a ribosome-binding site can be positioned 5' of the start codons. One or more cloning sites can be located 3', 5', or 3' and 5' of the portion encoding histidine tags to facilitate cloning. The vector can be, for example, the pHis6 vector.--

Replace the paragraph beginning at page 6, line 12, with the following rewritten paragraph:

C4
--In another embodiment, the invention provides an isolated nucleic acid having the sequence of SEQ ID NO:15.--

Replace the paragraph beginning at page 6, line 14, with the following rewritten paragraph:

C5
--In another embodiment, the invention provides an isolated nucleic acid having the sequence of SEQ ID NO:16.--

Replace the paragraph beginning at page 6, line 29, with the following rewritten paragraph:

ab --Figure 1 is a diagrammatic illustration of the 3-frame His-tag coding sequence and its location within the structure of the pHis4 vector. The 3-frame His-tag coding region is 93 base pairs in length and spans the region of nucleotides 196 to 283 (SEQ ID NOs:1, 21, 24, and 26, where SEQ ID NO:26 is the complementary strand). The protein translation for each of the three frames is shown below the nucleic acid sequence (SEQ ID NOs:20 (frame 1), 22-23 (frame 2), and 25 (frame 3)). Poly-histidine residues comprising the histidine tag of each reading frame are shown in bold. The MCS is located 5' to the 3-frame his-tag coding sequence at nucleic acid positions 283 to 299. The arrow indicates the direction of translation. The T7 promoter, used for expression of a protein that is cloned 3' of the MCS, is located at positions 299 to 402. Nucleotides 403-631 contain the 5' untranslated region of the *E. coli* ompA gene, obtained from the plasmid pTrip1EX, while the remaining region of the pHis4 plasmid, nucleotides 632-4603 and nucleotides 1-196, is derived from the pZL1 plasmid.--

Replace the paragraph beginning at page 7, line 25, with the following rewritten paragraph:

ax --Figure 5A is the sequence of part of the T7 promoter, the ribosome binding site, and the triple-ATG sequence in the ORF Rescue vector (SEQ ID NO:17).--

Replace the paragraph beginning at page 7, line 27, with the following rewritten paragraph:

ax --Figure 5B is a diagrammatic illustration of the ORF Rescue vector, pHis6. The 3-frame His-tag coding region is 118 base pairs in length (SEQ ID NOs:27, 30, 34, and 37, where SEQ ID NO:37 is the complementary strand). The protein translation for each of the three frames is shown below the nucleic acid sequence (SEQ ID NOs:28-29 (frame 1), 31-33 (frame 2), and 35-36 (frame 3)).--

Replace the paragraph beginning at page 9, line 12, with the following rewritten paragraph:

a9 --The nucleotide sequences of the invention allow for the translation of a histidine tag regardless of the reading frame used in the gene sequence that is upstream or downstream of the 3-frame His-tag DNA sequence. That is, the triplet code is capable of encoding histidine residues in any of the three reading frames. This is illustrated in the following example. Although many sequences can code for three or more histidine residues in all three reading frames, the following sequence is illustrative.

5' AAG CTT CAC CAC CAT CAT CAT CAC GCA TCA CCA CCA CCA CCA CGC
ATC ATC ATC ACC ATC ACC TCG AGC GTC ACA CTA GCT GAG TAA GCA
TGC 3' (SEQ ID NO:1)--

Replace the paragraph beginning at page 9, line 23, with the following rewritten paragraph:

a10 --In the first reading frame, i.e., if the first nucleotide in this sequence is considered the first nucleotide position of a codon, the translation of this sequence will be:

5' AAG CTT CAC CAC CAT CAT CAT CAC GCA TCA CCA CCA CCA
K L H H H H H A S P P P

CCA CGC ATC ATC ATC ACC ATC ACC TCG AGC GTC ACA CTA GCT
P R I I I T I T S S V T L A

GAG TAA GCA TGC 3' (SEQ ID NO:1)
E * A C (SEQ ID NO:20)--

Replace the paragraph beginning at page 10, line 1, with the following rewritten paragraph:

a11 --In the second reading frame, i.e., if the second nucleotide in this sequence is considered the first nucleotide position of a codon, the translation of this sequence will be:

5' A AGC TTC ACC ACC ATC ATC ATC ACG CAT CAC CAC CAC

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S F T T I I I T H H H H H

CAC GCA TCA TCA TCA CCA TCA CCT CGA GCG TCA CAC TAG CTG
H A S S S P S P R A S H * L

AGT AAG CAT GC 3' (SEQ ID NO:21)
S K H (SEQ ID NOs:22 and 23)--

Replace the paragraph beginning at page 10, line 13, with the following rewritten paragraph:

--And finally, in the third reading frame, i.e., if the third nucleotide in this sequence is considered the first nucleotide position of a codon, the translation of this sequence will be:

5' AA GCT TCA CCA CCA TCA TCA TCA CGC ATC ACC ACC ACC ACC
A S P P S S S R I T T T T

ACG CAT CAT CAT CAC CAT CAC CTC GAG CGT CAC ACT AGC TGA
T H H H H H H L E R H T S *

GTA AGC ATG C 3' (SEQ ID NO:24)
V S M (SEQ ID NO:25)--

Replace the paragraph beginning at page 22, line 1, with the following rewritten paragraph:

--The His-tag DNA sequence 1 (SEQ ID NO:1) has *HindIII* and *SphI* sites at the 5' and 3' ends, respectively. They are used for cloning into a vector. SEQ ID NO:1 has the following sequence:

5' AAG CTT CAC CAC CAT CAT CAT CAC GCA TCA CCA CCA CCA CCA
CGC ATC ATC ATC ACC ATC ACC TCG AGC GTC ACA CTA GCT GAG TAA
GCA TGC 3' (SEQ ID NO:1)--

Replace the paragraph beginning at page 22, line 7, with the following rewritten paragraph:

--For cloning into a vector, the His-tag DNA sequence 2 (SEQ ID NO:2) was synthesized with *KpnI* and *XhoI* sites at the 5' and 3' ends respectively. SEQ ID NO:2 has the following sequence:

Q14
5' GTA CCC ACC ACC ATC ATC ATC ACG CAT CAC CAC CAC CAC GCA
TCA TCA TCA CCA TCA CCT CGA 3' (SEQ ID NO:2)--

Replace the paragraph beginning at page 22, line 12, with the following rewritten paragraph:

--b. Sequences of the PCR primers and linkers used in vector constructions

Q15
Linker 1a: 5' CTG CAG CGG CCG CG 3' (SEQ ID NO:3)

Linker 1b: 5' CTA GGC GCC GGC GAC GTC TCG A 3' (SEQ ID NO:4)

Linker 2a: 5' CTA GCT GCA GAT ATC A 3' (SEQ ID NO:5)

Linker 2b: 5' AGC TTG ATA TCT GCA G 3' (SEQ ID NO:6)

ZL2: 5' CCA TCG ATC CGA GAT AGG GTT GAG T 3' (SEQ ID NO:7)

HT1 : 5' ACG AGC TCA GGC AGA GAC GA 3' (SEQ ID NO:8)

HT2: 5' ACG AGC TCG CAG AGA CGA CG 3' (SEQ ID NO:9)

ZL1: 5' CCT CGA GTC ACA CAG GAA ACA GCT AA 3' (SEQ ID NO:10)

ZL3: 5' GGC TAG CAG CTG TTT CCT GTG TGA 3' (SEQ ID NO:11)

ZL4: 5' GTG GAG CAT CTG GTC GCA 3' (SEQ ID NO:12)

ZL8: 5' GAG ATC TGC CAT AAC ATG TCA TCA TAG CTG TTT CCT G 3' (SEQ ID NO:13)

ZL10: 5' GAG ATC TGC CAT AAC ATG TCA TCA TAG CTG TTT CCT G 3' (SEQ ID NO:13)

T7 Linker: 5' CTA GCC GAA ATT AAT ACG ACT CAC TAT AGG GAG AC 3' (SEQ ID NO:14)

pHis6L: 5' TAT ACA TAT GGC ATG GCA TGG CCA CTG CAG GAT CCA CCA CCA TCA TCA TCA CGC ATC ACC ACC ACC 3' (SEQ ID NO:15)

a16
pHis6R: 5' GAC GTC GCA TGC TTA CTC AGC TAG TGT GAT GGT GAT GAT
GAT GGC CTA TGG TGG TGG TGG TGA TGC G 3' (SEQ ID NO:16)--

Replace the paragraph beginning at page 23, line 4, with the following rewritten paragraph:

--c. *The triple-ATG sequence and upstream region*

a16
5' TAATACGACTCACTATAGGGAGACCACAACGGTTTCCCTCTAG
AAATAATTTTGTTTAACTTTAAGAAGGAGATATACATATGGCATGGCATGGC
CA 3' (SEQ ID NO:17)

5' ATGGCATGGCATG 3' (SEQ ID NO:18).--

Replace the paragraph beginning at page 26, line 24, with the following rewritten paragraph:

a17
--A fragment from pZL1 was PCR amplified using the primers ZL4 and ZL8 (SEQ ID NO:12 and 13). The primers ZL4 and ZL8 were designed with a *NheI* site or a *BglII* site at the 5' end, respectively. The PCR product was digested with *NheI* and *BglII*, and then ligated into the pSlip2 vector that had been digested with *NheI* and *BglII*. The resulting vector is pSlip3. A fragment was obtained from pSlip3 by PCR using the primers ZL10 and ZL2 (SEQ ID NO:13 and 7). The resulting PCR product was digested with *BglII* and *HindIII*, then ligated back into *BglII/HindIII*-digested pSlip3 to generate pSlip4.--

Replace the paragraph beginning at page 27, line 1, has been amended as follows:

a18
--Plasmid pSlip4 was digested with *MunI* and *NheI*. A T7 linker, composed of these oligonucleotide sequences: 5' CTA GCC GAA ATT AAT ACG ACT CAC TAT AGG GAG AC 3' (SEQ ID NO:14) and 3' GG CTT TAA TTA TGC TGA GTG ATA TCC CTC TGT TAA 5' (SEQ ID NO:19), were synthesized. The linker, engineered such that the 5' terminus of each of the two strands either has a *MunI* or a *NheI* 5' cohesive overhang, was ligated with the *MunI/NheI*-digested pSlip4 vector to generate the pSlip7 vector.--